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Procedia Food Science 1 (2011) 1454 – 1458

Procedia Food Science

11th International Congress on Engineering and Food (ICEF11)

Antioxidant activity and phenolic content of extracts from different *Pterospartum tridentatum* populations growing in Portugal

Maria Teresa Coelho[a], José Carlos Gonçalves[a], Vitor Alves[b], Margarida Moldão-Martins[b]*

[a]*Escola Superior Agrária de Castelo Branco, Quinta Sra de Mércules, Apartado 119, 6001-909 Castelo Branco (mteresacoelho@ipcb.pt)*

[b]*CEER – Biosystems Engineering. ISA. Technical University of Lisbon. Tapada da Ajuda. 1349-017 Lisboa, Portugal (mmoldao@isa.utl.pt)*

Abstract

In the present study, aerial parts of *Pterospartum tridentatum* plants, collected in three locations in Portugal, at different vegetative stages, were evaluated for their total phenolic content and the antioxidant activity of aqueous extracts. The influence of the seasonal variation in the yield and composition of the extracts was evaluated, in order to select the most appropriate harvest season. Among the populations assayed, the extraction yields have some differences with the harvest period but the highest yield extraction was obtained in the flowering season, using flowers (19.4g/100g plant dry mass) and the lowest extraction yield was also obtained in the same period but using stems. The antioxidant activity of the solid extracts of *Pterospartum tridentatum* aerial parts, was determined by the radical scavenging activity method using 2,2-diphenyl-1-picrylhydrazyl radical (DPPH). The greatest DPPH radical scavenging activity was observed in the flowering period (3.6 mMTrolox/Kg dry mater), but no significant differences for the dormancy period. According to these results, we can choose the harvest season more favorable. The total phenolic content (TP) of the extracts was evaluated by measuring the absorbance at 280 nm and the values ranged from 270.7 to 402.9 mg gallic acid equivalents per g dry matter. The vegetative stage did not influence this total phenolic content. From preliminary experiments, it is also anticipated a significant antimicrobial activity of the solid extracts against bacteria and fungus

Selection and/or peer-review under responsibility of 11th International Congress on Engineering and Food (ICEF 11) Executive Committee.

Keywords: Pterospartum tridentatum; aqueous extracts; extraction yield; antioxidant activity; phenolic content

* Corresponding author. Tel.: +351-21-3653547
E-mail address: mmoldao@isa.utl.pt.

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doi:10.1016/j.profoo.2011.09.215

1. Introduction

Pterospartum tridentatum L. Willk. [=*Chamaespartium tridentatum* (L.) P. Gibbs.; *Genista tridentata* L.] is an European endemic Leguminosae (=Fabaceae) species belonging to the subfamily Papilionoideae [1] and known as *carqueija* or *carqueja* in Portugal. This small shrub, growing up spontaneously to 100 cm, is very common in the mountains of the north of Portugal, showing yellow flowers, alternate branches and coriaceous winged stems [2].

The shrubs of *Pterospartum tridentatum* can usually be found in the understory of *Arbutus unedo, Pinus pinaster* and *Eucalyptus* forests and in abandoned lands with acidic soils. Since ancient times, spices and herbs have been added to different food systems to improve flavors and for their antioxidant or antimicrobial capacity. Some authors refer the use of *Pterospartum tridentatum* in popular medicine for colds, stomach aches, intestinal problems, kidney disease, liver and bladder problems and also for rheumatism. It is also indicated for pneumonia, bronchitis and tracheitis, for headaches, coughs, to lower blood pressure and cholesterol levels, diabetes and even weight loss programs. It is also used in cooking, as a condiment in rice and rabbit stew. The shrub is known for its diuretic, purgative, emollient, laxative, hypotensive, hypoglycemic and digestive properties. Bioactive compounds, such as alkaloids and flavonoids, have been identified in aqueous extracts of those plants [3].

Plants synthesize compounds with biological activity, namely antioxidant, as secondary products, which are mainly phenolic compounds serving in plant defense mechanisms to counteract reactive oxygen species (ROS) in order to avoid oxidative damage. Natural antioxidants against the protection of foods can also protect the human body from free radicals and the advancement of certain chronic diseases [4] and is often presumed to be safe for consumption, due to their plant origin [5], but this may vary depending on the plant species and environmental factors that affect growth.

Natural antioxidants present in fruits, vegetables, herbs and species, phenolic compounds (flavonoids, phenolic acids and tannins), nitrogen containing compounds (alkaloids, amino acids, peptides and amines), carotenoids, tocopherols or ascorbic acid and its derivatives [6], have been reported to be potential candidates in lowering cardiovascular diseases [7] and anticarcinogenic activities [8;9] and the phenolic compounds can exhibit antioxidant, antiallergenic, antiarthrogenic, antiinflamatory, antimicrobial and antithrombotic effects [10]. On the other way, researchers are looking for natural antioxidants as alternative to synthetic antioxidants, such as butylated hydroxytoluene (BHT), butylated hidroxyanisole (BHA) and *ter*-butylhidroxyquinone (TBHQ) that are widely used in the food and pharmaceutical industries. Interest in plant-derived food additives has grown, because the consumption of synthetic antioxidants has been related to the possible health risks, so it resulted in strict regulations over their use in foods.

Carqueja is an underexploited natural source of compounds with biological activity, which should be fully characterized aiming to its valorization.

The aim of the present study was to evaluate the total phenolic content and antioxidant activity of aqueous extracts of *Pterospartum tridentatum* samples, collected in three locations in Portugal, and in different vegetative stages.

2. Materials & Methods

Samples of the aerial parts of *Pterospartum tridentatum* were collected at different vegetative stages: dormancy period (end of January) and flowering period (in May). The shrubs were collected in three locations in Beira Interior, Portugal: Orvalho, Gardunha mountain and Malcata mountain. The characterization of harvesting areas is shown in Table 1, indicating the altitude and the means annual precipitation and temperature and also the type of soil where plants were harvested (Table 1).

Table 1. Harvest locals in Beira Interior, Portugal

Local harvest	Altitude (m)	Precipitation (mm)	Temperature (ºC)	Soil type
Orvalho	624	1200 to 1400	7.5 to 10.0	Lithosol and Cambisol
Gardunha mountain	916	1200 to 1400	7.5 to 10.0	Cambisol
Malcata mountain	743	800 to 1000	12.5 to 15.0	Lithosol and Cambisol

Aqueous extractions were performed by refluxing during 2 hours a mixture of 50 g of plant samples and 250 mL of water in a Clevenger apparatus. The extract solutions were freeze-dried and a solid extract was recovered.

The antioxidant activity of the solid extracts was determined by the radical scavenging activity method using 2,2-diphenyl-1-picrylhydrazyl radical (DPPH). After 40 min incubation period at room temperature in the dark, the absorvance was measured at 515 nm. The radical scavenging activity (%) was calculated as follows: RSA (%) = $[(Abs_{control} - Abs_{sample})/ Absb_{control}] \times 100$, where $Abs_{control}$ is the absorvance of DPPH radical in methanol and Abs_{sample} is the absorvance of DPPH radical in the sample extract. Results were expressed as Trolox equivalents (mMtrolox/Kg of dry mass).

The total phenol content (TP) of the extracts was evaluated by spectrophotometric method, measuring the absorbance at 280 nm. Total phenol values were expressed as gallic acid equivalents (mg/g of dry mass).

All trials were carried out in triplicate. The data were subjected to one-way analysis of variance (ANOVA) and the differences between means were measured using Duncan's Test through STATISTICA, Version 7 (Copyright© StatSoft, Inc.), p values < 0.05 were considered to be significant.

3. Results & Discussion

The influence of the seasonal variation in the yield and composition of the extracts was evaluated, in order to select the most appropriate harvest season. The extraction yields presented some differences with the harvest period (Table 2). Among the populations assayed the highest yield extraction was obtained in the flowering period, using flowers (19.4g/100g plant dry mass in Gardunha mountain) and the lowest extraction yield was also obtained in the same period but using stems (11.3 g extract/100 g plant dry mass in Malcata mountain). At the flowering period, the extraction yield is significantly different if it is used stems or flowers.

Table 2. Extraction yield by local, harvest period and part of the plant (g extract/100 g plant dry mater)

Local harvest	Harvest Period		
	Dormancy period/Stem	Flowering period	
		Stem	Flower
Orvalho	15.8 ± 0.93 a	12.8 ± 0.56 b	16.8 ± 1.56 a
Gardunha	15.7 ± 1.37 a	14.9 ± 0.83 a	19.4 ± 1.22 c
Malcata	18.9 ± 1.31 c	11.3 ± 1.21 b	14.8 ± 0.26 a

Data are the means ± standard deviation of three replicates. Mean value followed by different letters indicate significant statistical differences ($p < 0.05$).

The extraction yield was always higher than for the commonly used herbs. *Pterospartum tridentatum* plants gives rise to higher yields than other shrubs, like *Erica spp.* and *Cytisus scoparius* [11].

The 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) is widely used to evaluate the antioxidant capacity of extracts from different plant materials. It is used for measuring the ability of the extracts or pure

molecules to scavenge free radicals. In the DPPH test, the antioxidants were able to reduce the stable DPPH radical to the yellow-coloured diphenyl-picryl hydrazine. The effect of antioxidants on DPPH radical-scavenging was conceived to be due to their hydrogen-donating ability. As shown in Table 3, regarding the stem extracts, significant differences were found between the dormancy and the flowering period. At flowering stage a higher antioxidant activity was observed in the flower extracts.

Table 3. Antioxidant activity of aqueous extracts of *Pterospartum tridentatum* in DPPH radical scavenging activity (mMTrolox/Kg dry mater)

Local harvest	Harvest Period		
	Dormancy period /Stem	Flowering period	
		Stem	Flower
Orvalho	3.6 ± 0.07 a	3.2 ± 0.07 b	3.5 ± 0.08 a
Gardunha	3.6 ± 0.01 a	3.2 ± 0.07 b	3.6 ± 0.05 a
Malcata	3.6 ± 0.03 a	3.2 ± 0.14 b	3.5 ± 0.03 a

Data are the means ± standard deviation of three replicates. Mean value followed by different letters indicate significant statistical differences ($p < 0.05$).

Phenolics are plant secondary metabolites and are very important of their antioxidant activity and this is believed to be mainly due to their redox properties, which play an important role in absorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides [12]. The total phenolic content, ranged from 270.7 to 402.9 mg gallic acid equivalents per g dry matter (Table 4), and the highest value was observed at the dormancy period in plants from Malcata mountain.

Table 4. Total phenolics content of aqueous extracts of *Pterospartum tridentatum* (mg gallic acid equivalents per g dry matter)

Local harvest	Harvest Period		
	Dormancy period /Stem	Flowering period	
		Stem	Flower
Orvalho	331.7 ± 35.05 ab	320.0 ± 70.23 ab	270.7 ± 70.80 b
Gardunha	394.0 ± 74.45 a	335.9 ± 34.59 ab	315.8 ± 73.50 ab
Malcata	402.9 ± 17.07 a	337.7 ± 50.83 ab	309.5 ± 19.82 ab

Data are the means ± standard deviation of three replicates. Mean value followed by different letters indicate significant statistical differences ($p < 0.05$).

The total phenolic content of *Pterospartum tridentatum* shows very high levels at any time of harvest and are superior to other species previously studied, like *Harpephyllum caffrum* in leaf and stem bark and *Sclerocarya birrea* in stems, young stems and opercula [10]. They also contain much more phenolic compounds when comparable with results in *Carissa opaca* [13] or in Australia herbs and spices like *Tasmannia* pepper leaf, anise myrtle and lemon myrtle [14]. Plants with high levels of phenolic compounds have demonstrated a high antioxidant activity of plants extract. [15].

From preliminary experiments, it is anticipated a significant antimicrobial activity of the solid extracts against bacteria and fungus (data not shown).

4. Conclusion

The studied *Pterospartum tridentatum* aqueous extracts present a high extraction yield, an appreciable level of total phenolic compounds and a significant antioxidant activity. The results foresee a high

potential for the utilization of this plant or its extracts as a new source of safe natural antioxidants and preservatives for the food industry with consequent health benefits for consumers. From the results, it can be conclude that the plants can be harvested at all seasons of the year, which presents an advantage from an industrial point of view.

Acknowledgements

The authors acknowledge the research grant PROTEC from Fundação para a Ciência e Tecnologia (SFRH/PROTEC/67682/2010)

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Presented at ICEF11 (May 22-26, 2011 – Athens, Greece) as paper FPE1149.